EVALUATION REPORT ON ZECTRAN[R]

A Substitute for DDT in the Control of Western Spruce Budworm

by the staff of the

Insecticide Evaluation Project, Research Work Unit No. 2203

Pacific Southwest Forest and Range

Experiment Station

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In 1964, a new Insecticide Evaluation Project was established by the U.S. Forest Service at the Pacific Southwest Forest and Range Experiment Station in Berkeley, California. Its broad mission was to develop effective insecticides for major forest insect pests which would minimize environmental contamination. Two general approaches were to be followed: (1) find alternatives to conventional insecticides that were effective, nonpersistent, and specific or selective against the target insect; and (2) increase the efficiency of insecticidal treatments by improving formulations and application techniques.

A committee of Forest Service representatives agreed that the initial target insect would be the western spruce budworm, Choristoneura occidentalis. At the time, this insect was widespread throughout the Intermountain Region and parts of the Pacific Northwest and is considered to be the most serious defoilator.

This report is an attempt to summarize the research on Zectran conducted by the Insecticide Evaluation Project in Berkeley since 1964, and to document the results of other research which have a bearing on the safety of Zectran for use in the forest environment.

Since about 1949, epidemics of the spruce budworm were controlled in this country by aerial spraying with DDT--usually a dosage of 1 pound of insecticide per acre in a liquid formulation. With evidence mounting about the enviromental hazards of DDT--mainly its persistence in the environment and its tendency to build up in food chains--the Forest Service was anxious to develop a safer method for controlling spruce budworm. Because research on radically new methods of control might be excessively time consuming or unproductive, it was decided that the project should consider only chemicals already in production or experimental compounds near the production stage. Likewise, the compound selected should be applied with conventional equipment and procedures that had been developed for aerial spraying with DDT.

Scientists with the Insecticide Evaluation Project (including entomologists, chemists, plant physiologists, and other specialists), believed that an effective treatment could be developed if three conditions were met: (1) the insecticide should be more toxic to spruce budworm than to other organisms; (2) the insecticide and its breakdown products must not accumulate in any plant or animal system in the forest ecosystem and (3) the insecticide should be directed to the target insect with a high degree of efficiency.

In initial laboratory tests, the carbamate insecticide Zectran®, manufactured by the Dow Chemical Co., was 20 to 25 times more toxic to the budworm than DDT. This meant it could be used in much smaller quantities with less risk of damage to other forest insects and wildlife. The parent compound and its metabolites were readily broken down by exposure to air,

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In initial laboratory tests, the carbamate insecticide Zectran®, manufactured by the Dow Chemical Co., was 20 to 25 times more toxic to the budworm than DDT. This meant it could be used in much smaller quantities with less risk of damage to other forest insects and wildlife. The parent compound and its metabolites were readily broken down by exposure to air,

sunlight and in plant and animal systems. Although Zectran has a relatively high oral toxicity to mammals, it has a much lower dermal and chronic feeding toxicity--the main potential hazards in field use.

Field tests from 1964 to 1969, described in detail in a section of this report, showed that Zectran met the first and second conditions for a suitable insecticide better than any other candidate compound. Zectran also proved safe for handlers using it under field conditions. With these important criteria met, attention was directed to condition three--the task of directing the spray with greater efficiency to the target insect than to other organisms in the forest environment. This posed some major problems.

One of the major problems in distributing a pesticide from the air is penetration of the forest canopy. Any vegetation acts as a filter, but coniferous forests are especially efficient in filtering out the larger drops of a conventional aerial spray which may range from less than 1 to more than 350 microns. Approximately 75 percent of the volume of the spray drops are larger than 100 microns and do not penetrate the forest canopy.

Field tests conducted in 1965 and 1966 clearly indicated that only drops below 50 microns in diameter reached the budworm larvae with any degree of efficiency. This suggested that only about 5 percent of the volume of a conventional spray is effective against the spruce budworm. The remaining 95 percent does not reach the target insect and contaminates the enviroment.

Considerable effort from 1967 to 1969 was put into developing application techniques and equipment that would produce a higher proportion of small droplets. The goal was to produce droplets less than 50 microns

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in size. Although progress in reducing droplet size was made by the Forest Service Equipment Development and Testing Center at Missoula, Montana, problems were still encountered in distributing the spray distributed effectively within the forest canopy.

In 1969, because of the magnitude of the problem, the job of developing application techniques and special equipment for aerial spray operations was transferred to a new research unit at the Pacific Northwest Forest and Range Experiment Station at Corvallis, Oregon. Developing effective and efficient spray systems for applying Zectran or other compounds for forest insect control will be the responsibility of this research unit in the future.

After 5 years of research and field testing, Zectran has been recommended for use against the spruce budworm by representatives from the U.S. Forest Service Division of Pest Control, State & Private Forestry, Research and Equipment Development. Next, the insecticide and the system for its use must be registered by the Pesticide Regulations Division of the Agricultural Research Service.

PROPERTIES OF ZECTRAN

Zectran, chemically 4-dimethyl-amino-3,5-xylyl methyl-carbamate ($C_{12}H_{18}N_2O_2$), is a product of the Dow Chemical Company. In the past, it has been registered and sold for use against snails and insects on ornamental plants. Recently, it has been taken off the market by Dow because of the demand for its use in research programs.

Zectran belongs to a group of insecticides known as carbamates which in general have one very desirable property for an insecticide. Animals exposed to them have a very rapid recovery from cholinesterase inhibition. Cholinesterase activity is necessary to the normal transmission of impulses in animal nervous systems. Insecticides affect the transmission of nerve impulses in such a way that the transmission is continuous. In Zectran, this effect is of very short duration and is rapidly reversed. (1) Recovery from carbamate induced cholinesterase inhibition produced by carbamates is quite rapid, within a half hour. This indicates that Zectran would not produce a cumulative cholinesterase inhibition as is the case with most organophosphorous insecticides.

Zectran also has some desirable properties which distinguish it from many other carbamates. In field application it is selective against the western spruce budworm, being more toxic to the budworm than to most other forest insects, and it has very low persistence in the environment.

Zectran is a dry, white crystalline solid at 85°C. It is slightly water soluble, but does not go into solution readily in most commonly-used spray solvents. However, it is quite soluble in glycal ethers.

LABORATORY TESTS

Toxicity

Spruce Budworm

The toxicity of Zectran to the western spruce budworm larvae has been determined by applying the insecticide topically (on the surface) to the 6th instar. Zectran proved unusually toxic to the budworm--in fact, 20-25 times more toxic than DDT (fig. 1). Zectran has shown the same level of activity against spruce budworm as pyrethrins, a compound which has almost invariably been the most toxic candidate in tests on 19 species of lepidopterous insects. (2, 3, 4) Both sexes of spruce budworm are equally affected (table 1).

The toxicity of Zectran as a spray was determined in a laboratory spray chamber using 6th instar larvae. The dosage needed to produce 90 percent kill in the spray chamber was:

	ounces per acre
Zectran	0.66
Pyrethrins	0.54
DDT	14.00

Populations of western spruce budworm from both Montana and New Mexico were tested and found equally susceptible to Zectran sprays in the laboratory.

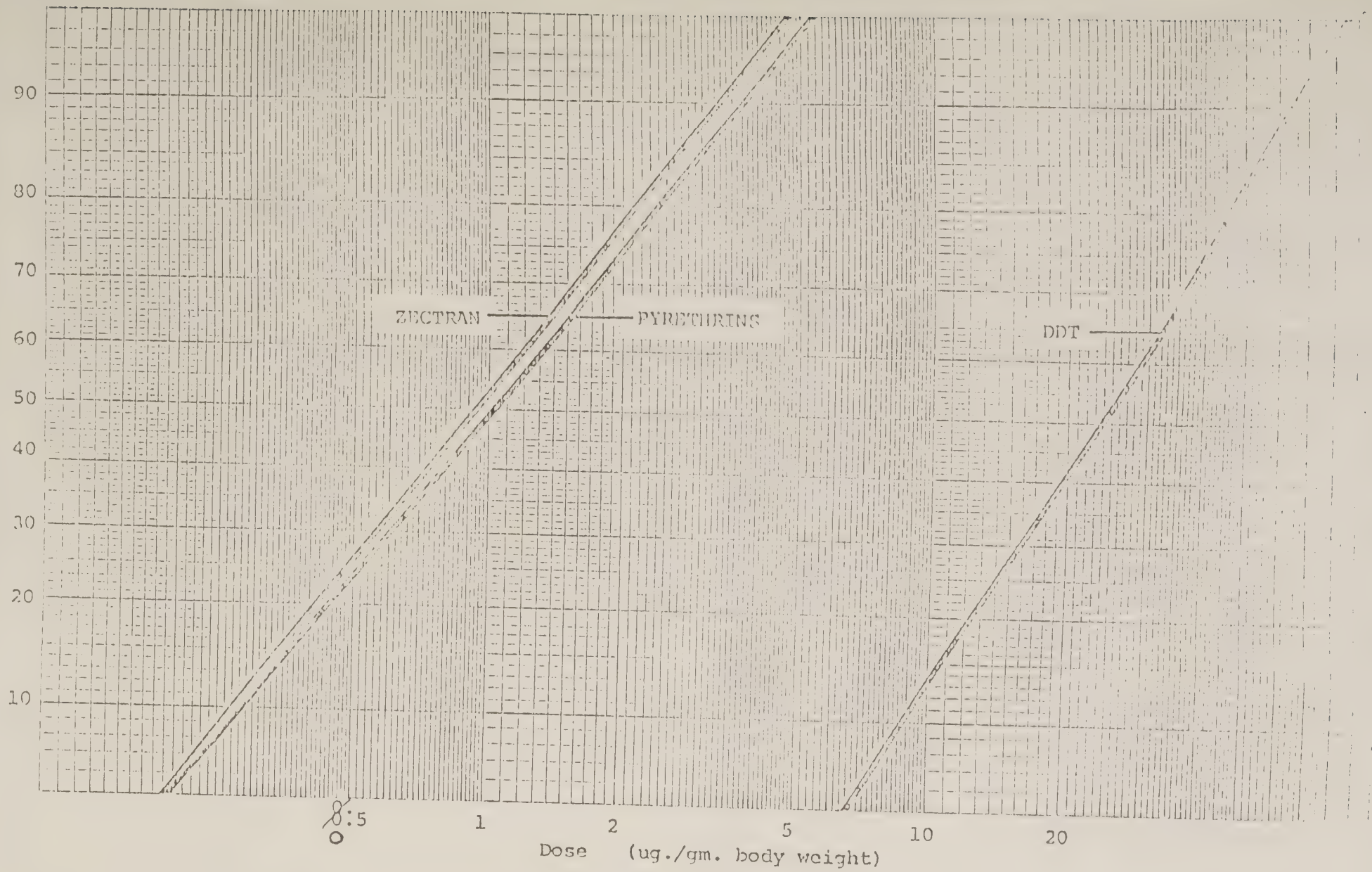

Figure 1--Toxicity of Zectran, pyrethrins, and DDT to western spruce budworm by topical application.

Table 1--Toxicity of Zectran topically applied to 6th instar western spruce budworm

Sex	LD/50[2/]	LD/90
	---ug./gm. body weight[3/]---	
Males	0.42	1.74
Females	.42	1.39

1/ Formulated in acetone and applied at rate of 1 ul./insect. Insects held in petri dishes without filter paper.

2/ Ld/50 refers to that amount of insecticide required to kill 50 percent of the test population. LD/90 refers to the amount required to kill 90 percent of the test population.

3/ 7-day post-treatment mortality observations.

Tests of sprays against larvae in various stages of development showed Zectran to be slightly more toxic to the 2nd, 3rd, and 4th instars than to the 5th and 6th instars (table 2). The toxicity of Zectran to the life stages in descending order was: Adults > larvae (6th instar) > pupae.

Oral toxicity of Zectran to the spruce budworm was higher than pyrethrins by 1.7 to 2.6 times and was 2.3 to 5.3 times higher than DDT (table 3).

Thirty-nine compounds have been tested to check their capacity to synergize (increase the toxicity of) Zectran. Only two showed even a low level of synergism. None can be considered promising enough to be used in spruce budworm control.

Fish, Birds, and Wildlife

The acute oral, chronic feeding, and dermal toxicity of Zectran has been thoroughly investigated by Dow Chemical Co., the U.S. Department of Interior at its Patuxent and Denver Laboratories, the U.S. Forest Service, the Department of Entomology of the University of California at Berkeley, and Russian scientists. This work is summarized in Table 4.

The acute oral toxicity of Zectran for rats ranges from 13-65 milligrams per kilogram of body weight (mg./kg.). This means Zectran has a relatively high oral toxicity for mamals. Care must be exercised in its use to be sure that the chemical is not swallowed. The dermal toxicity, however, is extremely low, indicating that Zectran does not present a hazard from handling or repeated contact. Dermal toxicity was nil at 2,000 mg./kg. for rabbits.

Table 2--Toxicity of Zectran spray[1/] to instars of spruce budworm

Instar	Mortality at 7-day post-treatment count[2/]
	percent
2nd	42
3rd	43
4th	32
5th	20
6th	16

1/ Zectran formulated at 0.15 mg./ml. in kerosene; Dowanol EB (9:1); mass median diameter of spray droplets was 90 microns.

2/ Application rate was 3 gal./acre with a Potter tower.

Table 3--Oral toxicity of Zectran pyrethrin and DDT[1/] to 6th instar western spruce budworm

Insecticide	LD/50	LD/90
	---ug./gm. body weight[2/]---	
Zectran	4.6	17.5
Pyrethrins	12.2	30.1
DDT	24.4	40.2

1/ Insecticides fed to larvae in artificial diet. Diet fed as a plug in glass tubing in which insects were confined individually. Analyses showed no breakdown of Zectran in diet.

2/ Mortality observations made 7 days after treatment.

Table 4--Toxicity of Zectran

Test animal	Acute oral	Intraperitoneal
	--- mg./kg. ---	
Albino rat	13 - 65[1/] 14.14[2/]	
Swiss mouse	30 - 50[3/]	
white rat		22.5[4/]
white mice		195.0[4/]
Lesser sandhill crane	1.0 - 4.5[2/]	
Canada goose	2.64	
Mourning dove	2.83	
Mallard duck	3.00	
Coturnix quail	3.21	
Mallard duckling	4.20	
Ring-necked pheasant	4.50	
House finch	4.76	
Chukar partridge	5.24	
Domestic pigeon	6.47	
Sharp-tailed grouse	10.00	
Domestic goat	15.0 - 30.0	
Mule deer	20.0 - 30.0	
English sparrow	50.40	
Bullfrog	283.0 - 800.0	

1/ Dow Chemical Company.

2/ U.S. Fish and Wildlife Service, Denver, Colorado. All figures that follow reported by this agency.

3/ Pacific Southwest Forest and Range Experiment Station, USFS Berkeley, California.

4/ Russian reports.

Studies by the U.S. Fish and Wildlife Service at Denver indicate that Zectran has no cumulative activity. For example, daily doses of Zectran producing symptoms of toxicity could be tolerated by mule deer for months without any permanent detectable effects. (See Tucker and Crabtree, EE 62:1307, 1969)

Since they have a very high metabolic rate, birds are very susceptible to the effects of many insecticides. The effects of Zectran have been carefully studied. Although oral dosages of 3.0 to 5.2 mg./kg. killed 50 percent of a test population of mallards and chukar partridge, these birds could tolerate 40 percent of an LD/50 for 30 days. (LD/50 refers to that amount of insecticide required to kill 50 percent of the test population.) Reproduction of the chukars was similar to that of the controls. (5)

Zectran is especially safe to fish. It is one of the least toxic chemicals tested on game fish by the U.S. Fish and Wildlife Service at their Fish Pesticide Laboratory in Denver. For example, the toxicity of Zectran to fish is much lower than that of DDT. The lethal concentration of Zectran required to produce 50 percent mortality in a test population of fish (LC/50), for example, is 5.3 mg./l. (milligrams per liter of water). For DDT, it is 0.002 mg./l. (6)

Zectran has also been tested on bullfrogs, which exhibit a high tolerance to the insecticide. LD/50 ranges from 283 to 800 mg./kg. (7)

The hazard to aquatic insects is also quite low, although the LC/50 is below 0.1 mg./l. (6)

Persistence

Zectran has been shown to be a very non-persistent insecticide, breaking down in sunlight within a few hours. In laboratory studies, the residual activity of Zectran and DDT was compared by spraying potted Douglas-fir trees with doses which were equivalent to 0.5 oz./acre of Zectran and 14 oz./acre of DDT. The deposits were allowed to age outdoors before caging insects on the treated trees. The residual life of Zectran was very short. Deposits were almost completely nontoxic after two days. The toxicity of DDT was essentially unchanged after the same length of time (table 5).

Laboratory findings have been backed up by studies in the field which also indicate no tendency for persistence or accumulation of Zectran, or its breakdown products, in the environment.

Residues of Zectran on Douglas-fir and five common browse plants were determined during field tests in Montana in 1966. Zectran levels dropped rapidly in most of the plants investigated after the first day (table 6). The amount left after one week was negligible except perhaps in the Frageria and Ceanothus species. All were insignificant within a month. (13)

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Table 5--Residual life of Zectran and DDT on potted Douglas-fir trees. Bioassay with 6th instar western spruce budworm[1/]

Aging period outdoors	Corrected mortality (7-day post-treatment count)	
	Zectran 0.5 oz./acre	DDT 14 oz./acre
hours	--- percent of insects killed ---	
0	90	98*
4	66	--
24	36	90*
48	7	95

1/Insects caged on foliage after deposit was exposed to sunlight outdoors.

Table 6--Zectran found after period indicated (PPM by species)

Species	Days								Weeks			Recovery factor
	0	1	2	3	4	5	6	8	2	3	4	
												(percent)
Pseudotsuga mensiesii	2.86	0.19	0.22	0.15	0.14	0.17	0.14	0.17	--	0.13	--	77
Balsamorhiza sp.	7.85	.47	.33	.28	.94	.82	.94	.13	0.22	.04	0.00	85
Ceanothus sp.	1.29	1.56	.94	1.25	1.52	1.56	1.00	.67	2.01	--	.19	48
Fragaria sp.	6.25	4.17	4.58	2.19	5.73	2.71	3.10	--	.73	.94	.38	48
Taraxacum sp.	2.29	.44	.11	.11	.08	.04	.01	.13	.07	--	0.00	70
Tragopogon sp.	.75	.56	.29	.06	.04	--	.04	.01	.01	--	.11	80

Breakdown Products

Like most insecticides which are nonpersistent, Zectran breaks down into other compounds, both in sunlight and in plants and animal systems. These breakdown products have been studied, both in the field and in the laboratory, to determine if they are toxic or persistent.

Photoxidation plays a strong role in the breakdown of Zectran. (10) Three hours exposure to ultraviolet light led to the production of 6 or more compounds that were shown to be cholinesterase inhibiting. More intensive breakdown would probably occur with additional exposure. This is backed up by the work of Abdel-Wahab and J.E. Carida (J. Agr. Food Chem. 15:479-487, 1967), who has found that Zectran completely disappears from the surface of bean plants in two days, degrading into 8 or more breakdown products. The rapid degradation of Zectran is accompanied by a corresponding disappearance of its metabolites.

The major metabolites were found to be methylamino, amino, methyl formamido, and the formamido Zectran. Three of these metabolites showed cholinesterase inhibition. Both methylamino and amino showed toxicity to rats in the same range as Zectran.

The toxicity of the four major breakdown products of Zectran of spruce budworm has been determined by topical application. All were substantially less toxic than Zectran (table 7).

The breakdown of Zectran has also been studied in some other insects (11), plants, and animals. Working with larval insects, Roberts (11), found that Zectran was 50 percent metabolized within 3 hours after topical application to the spruce budworm, tobacco budworm (*Heliothis virescens*) and housefly (*Musca domestica*).

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Table 7--Toxicity of Zectran and four breakdown products when topically applied[1/] to 6th instar western spruce budworm

Compound	LD/50	LD/90
	ug./gm. body weight [2/]	
Zectran	0.52	1.13
Methylamino Zectran	1.32	4.55
Amino Zectran	1.00	5.01
Methyl formamide Zectran	5.42	14.10
Formamido Zectran	10.70	372.00

1/ Formulated in acetone, except amino Zectran which formulated in acetone and water (1:1), and formamido Zectran in acetone and DMSO (1:1).

2/ Application rate: 1 ul./insect; mortality counts made 5 days after treatment. Held in petri dishes without filter paper.

Mode of Action

Insects

Considerable research has been conducted in an effort to determine when, where, and how Zectran is degraded in the insect. In larval insects, Zectran is 50 percent metabolized, or broken down, within 3 hours after topical application to the western spruce budworm, tobacco budworm, and housefly. (11) Because research has shown that insecticides are detoxified at other sites in other insects, scientists felt that this might also be the case with Zectran and the spruce budworm An attempt was made to produce metabolism of Zectran in tissues and subcellular fractions isolated from the spruce budworm and housefly larvae. With these tests, scientists hoped to explain some of the unusual observations they have made about the insecticidal activity of Zectran on spruce budworm larvae. For example, insects often exhibit symptoms of poisoning other than cholinesterase inhibition. Also, it is not possible to synergize Zectran on spruce budworm.

Almost every tissue and subcellular fraction of the spruce budworm larvae has been tested. With only one exception (table 8), scientists have not been able to produce the same rate and type of oxidative metabolism of Zectran found after topical application to live larvae. A partial list of the tests run with Zectran is included in Table 9. This list indicates that we have tested most of the *in vitro* systems found in the literature including poly bound oxidase etc. The oxidative enzumes tyrosinase or polyphenol oxidase have also been incorporated into the insect homogenates with still no metabolism of Zectran.

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We are led to the conclusion that metabolism (oxidation) of Zectran may occur only in or on the cuticle of the spruce budworm larvae. This oxidation may be independent of enzyme activity, but perhaps aided by some organic or inorganic constituent on the surface of the cuticle. Because it is especially important to determine exactly how the insecticide is broken down by the insect, research will continue on this problem.

Table 8--Breakdown of Zectran in homogenates of western spruce budworm larvae (with enzymes FAD and NADPH added)

Minutes	Recovery of Zectran and breakdown products			
	Zectran	Methyl amino Zectran	Amino Zectran	Others
		-- percent --		
		FAD		
15	88.4	7.3	2.8	1.4
30	87.8	5.8	3.3	2.8
45	90.5	6.4	1.6	1.3
60	81.9	9.2	4.6	4.2
75	68.0	12.2	5.8	13.9
90	72.1	10.0	9.7	8.3
105	59.1	15.2	10.2	16.1
120	67.2	14.2	7.1	11.3
135	56.8	11.6	17.0	14.5
		NADPH		
15	29.5	64.8	0	3.0
30	33.7	61.6	0	4.7
45	37.0	58.0	0	4.9
60	38.2	56.8	0	5.0
75	23.7	73.0	0	3.3
90	26.1	71.5	0	2.3
105	4.2	91.3	0	4.5
120	30.2	60.4	0	9.6

Table 9--Tests conducted to determine breakdown of Zectran in preparations of western spruce budworm larvae and various cofactors

Preparation	Cofactors[2/]	Additives[1/]			
		ph 0.1 M sodium phosphate buffer	0.25 M sucrose	1.25 W/V Bovine serum albumin	Incubating time
					(hours)
Whole homogenate	FAD	7.2	X	X	2.25
	FAD & MgCl2	7.2	w & w/o	X	4.00
	NADPH	7.22.5			
		6.7			
		7.3			
		9.0	w & w/o	X	2.00 & 4.00
	NADPH & FAD	7.2	X	X	2.00
	ATP & $MgCl_2$	7.3	O	X	2.00
	NADP	7.3	O	X	2.00
Boiled whole homogenate	NADPH	7.3	X	X	2.00
Heads and thorax	NADPH & FAD	7.3	X	X	2.00
Thorax and abdomen	NADPH	7.6 & 7.3	X	X	2.00
Abdomens		9.0	O	O	2.00
Gut	NADPH & FAD	7.3	X	X	2.00
Fat body and hemolymph	NADPH or FAD	7.2 & 9.0	X	X	2.00
Hemolymph	NADPH	6.7	X	X	2.00
Cuticles	NADPH or FAD	6.7, 6.8 & 9.0	w & w/o	X	2.00

Cuticles after washing with 0.1 N Hcl	NADPH or FAD	6.4 & 6.7	O	X	2.00
Soluble fraction of whole homogenates after centrif. at 10,000 + g 10 min.	NADPH & NADP & FAD	7.2	w & w/o	X	2.00
Sediment (mitochrondial) after above centrif.	NADPH & FAD	7.2	X	X	2.00
Soluble fractions after 105,000 x g for 1 and 2-1/2 hrs	NADPH & FAD	7.2	X	X	2.00
Microsomal pellet	FAD w and w/o MgCl2	7.3	w & w/o	X	3.00
	NADPH w & w/o MgCl2	6.7			
		7.3	w & w/o	X	3.00

1/ Carbonyl labeled Zectran was added at 0.72 mm. at 6.3 sp. act. or approximately 200,000 dpm/flask. This concentration also varied from 0.18 to 1.44 mm. flask.

2/ Cofactors were usually added at 0.37 mm. concentrations, however, the concentration was varied from 2.0 um to 0.5 mm.

Plants

Considerable effort has gone into the study of the mode of action of Zectran in plants, especially in connection with research aimed at producing systemic insecticides.

Aerial application of an insecticide places the insecticide on the host plant. Entry into the plant is through the cuticle and cellular membrane, stomata, or bark. To achieve penetration through these tissues, a fat-soluble molecule is required. However, to acquire efficient translocation after the molecule has penetrated the epidermis, a water-soluble molecule is necessary. Zectran is soluble in water to the extent of 100 parts per million.

Penetration studies have been conducted with cuticular membranes isolated from white fir, Douglas-fir, ponderosa pine, apricot, agave leaves, and tomato fruit. Zectran is one of over thirty compounds which have been evaluated: (fat soluble) carbamates (e.g., Landrin®), penetrated readily, while Zectran penetrated more readily than the following: halogenated hydrocarbons, pyrethrins, organophosphates, triazines, pyridillium chlorides, substituted ureas, sucrose, and glucose. The thickness of the cuticle did not correlate penetration.

The movement of Zectran across or through isolated plant cells has also been studied in the laboratory. The data show that Zectran is absorbed in living cells by a diffusion process and is not actively pumped into the cell cytoplasm at concentrations greater than those in the surrounding solution or cell walls. Zectran diffuses across cell membranes more effectively than all the organophosphorous insecticides studied. However,

the more fat-soluble insecticides such as SD-8530 and DDT diffuse more rapidly because they are more fat soluble.

Three insecticides--Zectran, Matacil, and Bidrin--have been tested for their systemic activity under field conditions using spruce budworm as a bioassay organism. Research indicates that Zectran will not translocate with the sugar. The insecticides were injected into the trunks of Douglas-fir and grand fir trees of varying sizes. In Douglas-fir trees 3 feet or less in height, movement of Zectran was apparently good. High mortality of larvae resulted from an injection of the three compounds at rates of 40 and 200 mg. per tree. In trees 5 to 8 feet high treated with 0.2 to 1.0 grams of these insecticides, only Matacil and Bidrin yielded high insect mortality. Foliage and wood samples were analyzed for residues of Zectran. In the small trees, Zectran was found at 308 ppm, but not more than 20 ppm in the larger trees.

Analysis of wood samples near the point where Zectran was injected in the larger trees revealed concentrations as high as 8,460 ppm of Zectran. Immobility of Zectran within the tree is probably due to its partitioning into the oleoresin.

Gross autoradiography (a technique for study of translocation) with radioactive insecticides was used to study the translocation of various compounds in Douglas-fir, white fir, and ponderosa pine. Zectran, among others, does not accumulate selectively in certain tissues, but tends to be evenly distributed throughout areas of the tree where the water is moving (the xylem tissue).

The metabolism of 8 radioactive pesticides injected into young trees was studied in 2-year-old Douglas-fir, white fir, and ponderosa pine. The results after 30 days were as follows: For DDT 80 percent of the radioactivity was recovered as DDT, 12 percent as DDE, with 8 percent unaccounted for. With Zectran, 60 percent of the applied dose was recovered as Zectran, 30 percent was found as degradation products. The fact that large quantitites of Zectran were found intact after 30 days appears to contradict the results of other research which indicates, for example, that Zectran completely disappears from bean plants in two days. The longer period of persistence is explained in this case by the fact that Zectran was injected into the tree and did not have a chance to be broken down in sunlight. Also, in trees, the rate of metabolism is slower because Zectran is moving in the dead wood of the tree, and is not actively metabolized by living cells.

In an effort to understand how Zectran and other insecticides affect living tissue, scientists have also studied its effects on living cells, particularly on oxygen uptake. Tissues of the following were tested: yeast, beef heart, chlorella, bean hypocotyl, He-La, cells and guinea pig liver. At a dosage equivalent to 12 times that recommended for field use (0.15 lb./acre) Zectran definitely inhibited respiration at the cell level. This means that cholinesterase inhibition is probably not the only mode of action of the insecticide. The metabolites of Zectran were not found to inhibit oxygen uptake. However, analogs of Zectran, SD-8530, and Matacil were found to be almost as effective as Zectran.

Physiological Effects

As indicated elsewhere in this report, the oral toxicity of Zectran to mammals is quite high. The dermal toxicity of Zectran to mammals, however, is very low, and normal handling precautions would be sufficient to safeguard those working with it.

If Zectran is applied in the manner recommended in this report, the chances of its reaching human beings in quantities large enough to produce damage of any kind are extremely unlikely. However, no discussion of the safety of an insecticide would be complete without an analysis of its potential for producing cancers or birth defects. The authors of this report have conducted no research in this area. The following conclusions have been drawn from the available literature.

(Someone should summarize available literature on carcinogenic potential of Zectran.)

During the period 1965-68, the Bionetics Research Laboratories of Litton Industries, under contract to the National Cancer Institute, tested various pesticides and related compounds for possible teratogenic effects (birth defects). Zectran is one of a number of compounds tested by injecting the insecticide beneath the skin of mice. When applied at a rate of 10 mg./kg. of body weight in a DMSO solvent, Zectran produced no significant increase of anomalies in the two strains of mice tested. (12)

FIELD TESTS

The first small-scale field test of Zectran against western spruce budworm was conducted on the Salmon National Forest in Idaho during the summer of 1964. Three 20-acre plots were sprayed at the rate of 0.1 lb./gal. of cycle (petroleum) oil per acre. Results in terms of insect mortality were very promising, resulting in a 88 to 92 percent reduction of the insect population.

In 1965, Zectran was tested again in the West Fork Ranger District of the Bitterroot National Forest of Montana. Zectran was to be applied over 1,080 acres at the rate of 0.15 lb./gal./acre in a mixture of one part Zectran concentrate to nine parts cycle oil. Actual release from the fixed-wing aircraft was calculated to be 0.73 gal./acre. This resulted in a 91 percent reduction of the insect population.

In conjunction with the 1965 test, an additional 335 acres were sprayed with the same Zectran formulation but with fluorescent particles added. This was done in an attempt to measure the drop size reaching the insects and to determine how well the insecticide was being distributed. This test resulted in a population reduction of 98 percent.

In early spring of 1966, a test was conducted in the drainage of the West Fork of the Bitterroot River, also in the Bitterroot National Forest. There were several objectives: (1) to test Zectran at 0.15 lb./gal. per acre against western spruce budworm under normal spraying conditions in the field; and (2) to allow scientists to try new methods for determining spray distribution and budworm mortality; and (3) to monitor the effects of Zectran on several forms of wildlife, including small mammals, song birds,

grouse, fish, and other aquatic organisms. Two spray areas were selected, one 3,538 acres in size, the other 1,190 acres. Two check areas of 1,000 acres each were also established. Spraying was accomplished with a fixed-wing aircraft. The formulation consisted of one part Zectran/Dowanol mixture diluted in nine parts deodorized kerosene. This produced the desired dosage of 0.15 lb./gal./acre of Zectran. Fluorescent particles were also used in this test.

The 1966 test was evaluated by two separate sampling plans. One, using 50 one-tree plots throughout both sprayed areas, indicated an 87 percent population reduction in the 3,538-acre area and a 77 percent population reduction in the 1,190-acre area. A corresponding 44 and 49 percent population reduction occurred in the check areas. A second evaluation was made with the cluster-sampling method (used in previous years) in the 3,538-acre area. In this case, a 94 percent population reduction was indicated. (8)

In 1967, two spray tests were made. One was in the Sawtooth National Forest, Idaho, the other in northern Maine. Due to a catastrophic decline in the spruce budworm population during the spring in the Sawtooth area no reliable data was obtained on population reduction. However, the test in Maine gave valid results. The formulations consisted of 75 pounds of Zectran dissolved in 50 gallons of Dowanol TPM. This mixture was applied as a fine spray at the rate of 13 fluid ounces per acre, still 0.15 pounds of Zectran per acre. Approximately 500 acres were treated resulting in an 82 percent reduction.

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The foregoing generally outlines the high effectiveness of Zectran applied at 0.15 lb./acre. Subsequent tests have been conducted varying the dosage or the method of application to try to further reduce the dosage of insecticide.

During 1968, a test was conducted against the spruce budworm in Belmont and Chamberlain Creeks in the Blackfoot River drainage east of Missoula, Montana. Zectran was applied at a rate of .063 oz./acre in .25 gallon Dowanol with a droplet size no larger than 120 microns. Spruce budworm mortality was 70 percent in the Belmont Creek area. Insect mortality in the Chamberlain Creek areas was very low. The reasons for this are not completely understood. When using ultra-low-volume application or with very tiny spray droplets, air currents play a major role in distribution of the insecticide. A better understanding of the meteorological conditions in mountainous regions will be essential in order to take full advantage of atmospheric transport and dispersion in delivering the spray to the insect.

In 1969, another test of Zectran was carried out in the Nez Perce National Forest in Idaho. Two areas were selected, each about 4,000 acres. One was sprayed at a rate of 0.15 pounds of Zectran in 0.5-gallon of TPM. The other area was sprayed twice with 0.075 pounds of Zectran in 1/2-gallon of TPM. Results indicated less than 80 percent mortality in both areas. There was no significant difference between the plot sprayed once and the area which had been sprayed twice.

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ENVIRONMENTAL EVALUATION

Only a small fraction of the pesticides used in the United States are applied to forest lands. Yet forests are an especially important part of the total environment. They are the sources of major water supplies and home for most of our wildlife. The application of pesticides to forest lands must be done with great care, and with as little effect as possible on other insects, fish, birds, and other wildlife.

In the course of field testing Zectran for operational use against the spruce budworm, scientists have also investigated its potential effect on the rest of the forest community. Intensive studies of the effects of Zectran on non-target insects, fish and wildlife, and its residual characteristics in soils and plant tissues have also been made.

Terrestrial Insects

Results of the 1965 and 1968 field tests showed that Zectran is more effective against western spruce budworm than other forest insects. Zectran reduced western spruce budworm populations to a much greater extent than most insects found associated with it in the different crown levels. (8)

Results of the 1965, 1966,1968 and 1969 field tests showed that parasitism by Apanteles fumiferanae, and in most cases by Glypta fumiferanae increased following treatment with Zectran. It is possible that the budworm parasitized by Apanteles and Glypta survive the Zectran treatment more readily than healthy larvae. (9)

Drop cloths were set up during the 1965 spray test in Tough Creek and Mud Creek to catch dead insects. Results of these tests support the

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Terrestrial Insects

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results obtained by sampling defoliator populations in the tree crown. Zectran was more effective against defoliators than was naled, another insecticide being tested. Zectran also apparently killed more spiders (non-specific predators) and aphid-mite predators than did naled. However, naled killed more sap feeders and saprophytic insects than Zectran.

Insect traps designed to catch flying terrestrial insects were set up in Trapper Creek and Violet Creek in 1966 and 1967, and in Tough Creek (1965 field test) and One-two Creek in 1967. Terrestrial insects in Tough Creek appeared to be fully recovered from any effects of Zectran by 1967. The number and variety of insects were similar to those of unsprayed check areas.

Aquatic Insects and Fish

The effects of Zectran on fish and wildlife have been studied extensively both in the laboratory and in the field. During the 1965 Zectran test, the Bureau of Sport Fisheries and Wildlife undertook a study of the effects of Zectran on aquatic organisms. No conclusions can be drawn from this study because there was an increased drift of dead insects in the stream at all sampling stations, including the check stations, immediately after spraying. No direct effects of Zectran were observed on live-caged trout. Fish reacted normally throughout the test.

In a study conducted in 1966 on the Salmon National Forest, there was no significant increase of emigration and intrastream movemet of fish. No effects on benthic aquatic insect numbers were observed; however, more insects were observed drifting downstream about three hours after spraying.

This continued for several hours. Adult terrestrial insects, immature aquatic insects (Heptageneidae, and Phyacopheladae, and Blepheracendae) increased in drift samples after spraying. The conclusion is that Zectran damages aquatic organisms less than DDT, malathion and diazinon.

Further studies of the effect of Zectran on aquatic insects were conducted in Main during the 1967 tests. No significant change in insect numbers was evident in either bottom samples or drift samples following spray. Zectran had no observed effect on live-caged Eastern brook trout. No dead fish were observed in blocking nets. These results agreed with those obtained in the 1967 test.

Birds and Mammals

Extensive studies of the effects of Zectran on wildlife were conducted by the Bureau of Sport Fisheries and Wildlife, U.S. Department of Interior. In a 3-year study beginning in 1965, their work showed that Zectran had no discernable effect on birds or small mammals. In general, their observations indicated that: (1) no harm to birds or mammals resulted from the Zectran applications; (2) the spray temporarily increased the availability of budworm larvae and other insect food for birds; and (3) that the reduction in available food that followed this increase was not sufficient to cause birds to abandon their nest or enough to interfere with rearing of the young. The Bureau of Sport Fisheries and Wildlife concluded that over a period of three years, Zectran . . . "has shown no important detrimental effects to wildlife." (Richmond & Pillmore, 1967).

The Montana Fish and Game Department investigated the effects of Zectran on grouse for a 2-year period in an area sprayed in 1966. They

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also set up a special plot that was purposefully sprayed with 5 times the usual dose of 0.15 pound of Zectran per acre. With the use of banding, color marking, and radio telemetry, wildlife biologists were able to study subsequent movement of the birds. A summary of their results follows: (Morel et al.)

> The strongest evidence that Zectran did not affect blue grouse survival or behavior was obtained from the multiple-sprayed Mud Creek unit. Seven of 11 banded adult males, positively exposed to Zectran, survived to the following breeding season (10 months post-spray). An eighth banded male exposed to Zectran survived until fall (four months post-spray), when taken by a hunter.

Radio-telemetry showed that survival and behavior of seven brood hens in the heavily sprayed area was normal for 37 days following spraying. Repeated post-spray flush counts of the broods with instrumented hens gave no indication of chick mortality in six of seven broods. Chick loss occurred in one brood shortly after spraying; whether this was a direct effect of the pesticide or natural mortality is not known. A better evaluation of chick survival might be accomplished in the future by systematic flush counts of telemetered broods on both sprayed and unsprayed areas.

Some observations were also made from the Trapper Creek test (1966) where Zectran was applied at 0.15 lb./acre. The area was sprayed between June 30 and July 4. Only nine of the 45 males (blue grouse) on Trapper Creek study units are known to have been exposed to Zectran. Five of 21

females banded on Trapper Creek units were observed after spraying. Three of the five females were radio-equipped and monitored within the sprayed area for periods of from 7 to 19 days after application. The Trapper Creek study provided the only field evaluation for the effect of Zectran on ruffed grouse. Four of five banded males were observed to have survived for varying periods after the spray. There were no significant differences in the annual survival rates of banded blue grouse males on sprayed and unsprayed study areas. During this test, only 15 of the more than 75 blue grouse which had been banded in the same areas were located and identified. The only ruffed grouse located in the post-spray observation period were instrumented with transmitters.

None of the instrumented grouse died or showed signs of poisoning. The possiblity of finding pesticide casualties by telemetry was demonstrated by radio-tracking instrumented females to their nest sites. Eleven of the 13 instrumented grouse exposed to Zectran remained in sprayed zones for at least a week after spraying--the period when the relatively short-lived Zectran would be potentially the most hazardous. Limited telemetry studies did not reveal changes in respiration or activity pattern of instrumented grouse after they were exposed to aerially-sprayed Zectran.

Soils

Soil studies (See Teranee et al.) indicate that Zectran has no effect on microbial respiration when applied to soil as a dry powder, combined with soil litter, or applied in an acetone-oil carrier. Even when Zectran was applied to soil in concentrations far greater than 0.15 lb./acre, it had no significant effect on microbial activity. When properly applied, Zectran should pose no hazard to microbes in the soil.

NOTE ABOUT LITERATURE CITED

IEP project please complete those citations that have been left unfinished---#'s 5, 6, 7, and 10 on these two pages.

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